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FORM PTO-1390 (Rev 10-96) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 012627-003

U S APPLICATION NO (If known, see 37 C F R 1 5): 08/973,815

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE96/01016	10 June 1996	09 June 1995

TITLE OF INVENTION: DNASE-ACTIVE PROTEIN

APPLICANT(S) FOR DO/EO/US: Hanswalter ZENTGRAF; Annemarie POUSTKA; Johannes COY; Iris VELHAGEN

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☐ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☒ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☐ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. ☐ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☐ A copy of the International Application as filed (35 U S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US)
6. ☐ A translation of the International Application into English (35 U.S.C 371(c)(2)).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired
   d. ☐ have not been made and will not be made.
8. ☒ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98
12. ☒ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☐ A FIRST preliminary amendment.
    ☒ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☐ Other items or information:

04/07/1998 PVOLPE 00000026 08973815
01 FC:154 130.00 OP

(10/97)

U.S. APPLICATION NO (If known, see 37 C F R 1.50)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
08/973,815	PCT/DE96/01016	012627-003

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
Basic National Fee (37 CFR 1.492(a)(1)-(5)):		
Search Report has been prepared by the EPO or JPO $930		
International preliminary examination fee paid to USPTO (37 CFR 1.482) $720.00		
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) $790.00		
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO $1070 00		
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $98.00		
ENTER APPROPRIATE BASIC FEE AMOUNT =	$	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$ 130.00	

Claims	Number Filed	Number Extra	Rate		
Total Claims	-20 =		X$22.00	$	
Independent Claims	-3 =		X$82.00	$	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 130.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 130.00	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$	
TOTAL NATIONAL FEE =				$ 130.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 170.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 170.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

Teresa Stanek Rea
BURNS, DOANE, SWECKER & MATHIS, L.L.P.
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SIGNATURE

Teresa Stanek Rea
NAME

30,427
REGISTRATION NUMBER

(10/97)

80 Rec'd PCT/PTO 09 DEC1997

FORM-PTO-1390 (Rev. 10-96) U.S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: 012627-003

U S APPLICATION NO. (If known, see 37 C F R 1 5): 08/973815

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/DE96/01016	10 June 1996	09 June 1995

TITLE OF INVENTION
DNASE-ACTIVE PROTEIN

APPLICANT(S) FOR DO/EO/US
Hanswalter ZENTGRAF; Annemarie POUSTKA; Johannes COY; Iris VELHAGEN

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16. [ ] Other items or information:

U.S. APPLICATION NO (If known, see 37 C.F.R 1 50)	INTERNATIONAL APPLICATION NO. PCT/DE96/01016	ATTORNEY'S DOCKET NUMBER 012627-003

17. ☒ The following fees are submitted:	CALCULATIONS	PTO USE ONLY
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ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 930.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

Claims	Number Filed	Number Extra	Rate		
Total Claims	10 -20 =	0	X $22.00	$ --	
Independent Claims	2 -3 =	0	X $82.00	$ --	
Multiple dependent claim(s) (if applicable)			+ $270.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 930.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$	
SUBTOTAL =				$ 930.00	
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SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Teresa Stanek Rea
NAME

30,427
REGISTRATION NUMBER

(10/97)

09 APR 1998 #4

Patent
Attorney's Docket No. 012627-003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Hanswalter ZENTGRAF et al Application No.: Unassigned 08/973815 (Corresponds to PCT/DE96/01016) International Filing Date: 10 June 1996 For: DNASE-ACTIVE PROTEIN	Group Art Unit: Unassigned Examiner: Unassigned

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please amend the above-captioned application as follows:

IN THE CLAIMS:

Kindly cancel claims 8-10, without prejudice or disclaimer.

Kindly add claims 11 - 12:

--11. A method for the prevention or treatment of a condition involving apoptosis, said method comprising administering a protein of claim 1, an antibody directed to said protein or a DNA encoding said protein to a patient in need of such prevention or treatment.

12. A method for the diagnosis of a condition involving apoptosis, said method comprising using a reagent based on the protein as defined by claim 1, an antibody of said protein or a DNA encoding said protein to diagnose a condition involving apoptosis.--

REMARKS

Entry of the foregoing amendment is respectfully requested.

The claims have been amended to place them in better condition for U.S. patent practice.

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
Teresa Stanek Rea
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Date: April 3, 1998

Patent
Attorney's Docket No. 012627-003

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Hanswalter ZENTGRAF et al	Group Art Unit: Unassigned
Application No.: Unassigned (Corresponds to PCT/DE96/01016)	Examiner: Unassigned
International Filing Date: 10 June 1996	
For: DNASE-ACTIVE PROTEIN	

PRELIMINARY AMENDMENT

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please amend the above-captioned application as follows:

IN THE CLAIMS:

Kindly amend the claims as follows:

Claim 4, line 2, delete "or 3";

Claim 6, lines 1-2, delete "according to claim 1";

Claim 9, line 1, delete "or 3".

REMARKS

Entry of the foregoing amendment is respectfully requested.

The claims have been amended to eliminate multiple dependency and to place them in better condition for U.S. patent practice.

Application Serial No. PCT/DE96/01016
Attorney's Docket No. 012627-003

Should the Examiner have any questions concerning the subject application, a telephone call to the undersigned would be appreciated.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

By: _____
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Post Office Box 1404
Alexandria, Virginia 22313-1404
(703) 836-6620

Date: December 9, 1997

A Protein Having DNase Activity

The present invention relates to a protein having DNase activity, a DNA encoding the same and a process for the preparation thereof. In addition, the invention concerns the use of the DNA and the protein as well as antibodies directed against the protein.

It is known that many cells suffer from programmed cell death. This cell death is referred to as apoptosis. It is found e.g. in the case of organogenesis and metamorphosis, tissue atrophy and tumor regression. Apoptosis is accompanied by a condensation of cytoplasm, a loss of plasma membrane villi, a segmentation of the nucleus and particularly an extensive degradation of chromosomal DNA. The latter manifests itself in that a "ladder" of DNA fragments, particularly having a size of over 600 kb, 50-300 kb and 50 kb, is present in apoptotic cells. It has not been known by now which mechanisms are responsible for the degradation of chromosomal DNA. However, this would be necessary to better understand apoptosis so as to be optionally able to take measures for or against it.

Thus, it is the object of the present invention to provide a product by which the degradation of chromosomal DNA in apoptotic cells can be investigated.

According to the invention this is achieved by providing the subject matters as defined in the claims.

Therefore, the subject matter of the present invention relates to a protein having DNase activity, which comprises the amino acid sequence of fig. 1 or a functional derivative or fragment thereof.

The expression "DNase activity" refers to the fact that the protein can cut single-stranded and/or double-stranded DNA.

The expression "functional derivative or fragment" comprises any derivative or fragment of the amino acid

sequence of fig. 1, which has DNase activity. The amino acid sequence of fig. 1 may also include additions, substitutions and/or deletions of one or more amino acids, which also applies to the functional derivatives or fragments thereof.

A further subject matter of the present invention relates to a nucleic acid coding for an above protein. This may be an RNA or a DNA. The latter may be e.g. a genomic DNA or a cDNA. Preferred is a DNA which comprises the following:

(a) the DNA of fig. 1 or a portion thereof,
(b) a DNA hybridizing with the DNA of (a), or
(c) a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

The expression "hybridizing DNA" refers to a DNA hybridizing with a DNA of (a) under normal conditions, particularly at 20°C below the melting point of the DNA.

The DNA of fig. 1 was deposited with DSM (*Deutsche Sammlung von Mikroorganismen und Zellkulturen* [Germany-type collection of microorganisms and cell cultures]) as JFC4 under DSM 9993 on May 23, 1995.

A DNA according to the invention is described below in the form of a cDNA. It is exemplary for every DNA falling under the present invention.

For the production of a cDNA according to the invention it is favorable to use a cosmid library, e.g. q1Z (cf. Dietrich, A. et al., Nucleic Acids Res. 19, (1991), 2567-2572), as a basis, clones of which comprise the region Xq27.3-Yqter of the human genome. Such clones are fixed on a filter membrane and hybridized with labeled cDNA pools obtained from mRNA of pig tissues, e.g. brain, muscle, liver, by reverse transcription (cf. Coy, J.F. et al., Mammalian Genome 5, (1994) 131-137). Those clones having a hybridization signal with the cDNA pools are used for

screening a human cDNA library, e.g. of fetal cerebral tissue. For this purpose, particularly the cDNA library - Zap, Stratagene company, catalog No. 936206 is suitable. A cDNA according to the invention is obtained.

A cDNA according to the invention can be present in a vector and expression vector, respectively. A person skilled in the art is familiar with examples thereof. In the case of an expression vector for E. coli these are e.g. pGEMEX, pUC derivatives, pGEX-2T, pET3b and pQE-8, the latter being preferred. For the expression in yeast e.g. pY100 and Ycpad1 have to be mentioned, while for the expression in animal cells e.g. pKCR, pEFBOS, cDM8 and pCEV4 have to be indicated. The baculovirus expression vector pAcSGHisNT-A is especially suitable for the expression in insect cells.

The person skilled in the art knows suitable cells to express a cDNA according to the invention, which is present in an expression vector. Examples of such cells comprise the E. coli strains HB101, DH1, x1776, JM101, JM109, BL21, and SG 13009, the latter being preferred, the yeast strain saccharomyces cerevisiae and the animal cells L, 3T3, FM3A, CHO, COS, Vero and HeLa as well as the insect cells sf9.

The person skilled in the art knows in which way a cDNA according to the invention has to be inserted in an expression vector. He is also familiar with the fact that this DNA can be inserted in combination with a DNA coding for another protein and peptide, respectively, so that the cDNA according to the invention can be expressed in the form of a fusion protein.

In addition, the person skilled in the art knows conditions of cultivating transformed cells and transfected cells, respectively. He is also familiar with processes serving for isolating and purifying the protein expressed by the cDNA according to the invention. Thus, such a protein which

may also be a fusion protein also belongs to the subject matter of the present invention.

A further subject matter of the present invention relates to an antibody directed against an above protein and fusion protein, respectively. Such an antibody may be prepared by common methods. It may be polyclonal and monoclonal, respectively. For its production it is favorable to immunize animals - particularly rabbits or chickens for a polyclonal antibody and mice for a monoclonal antibody - with an above (fusion) protein or fragments thereof. Further "boosters" of the animals may take place with the same (fusion) protein or fragments thereof. The polyclonal antibody may then be obtained from the animal serum and egg yolk, respectively. For the monoclonal antibody, spleen cells of the animals are fused with myeloma cells.

A preferred antibody of the present invention, namely the monoclonal antibody 11/78/1, was deposited with DSM under DSM ACC 2211 on April 26, 1995.

The present invention enables to investigate the degradation of chromosomal DNA in apoptotic cells. This investigation can be carried out with a person's isolated body fluids. A DNase responsible for the above degradation can be detected by the antibody according to the invention. Furthermore, an autoantibody directed against this DNase can be detected by a protein according to the invention. Both detections may be made by common methods, particularly a Western blot, an ELISA, an immunoprecipitation or by immunofluorescence. In addition, the expression of the gene coding for the above DNase can be detected by a nucleic acid according to the invention, particularly a DNA and primers derived therefrom. This detection may be made as usual, particularly in a Southern blot.

Moreover, the present invention is suitable to take measures for or against apoptosis. These measures comprise the administration of a product according to the invention

to a person. An above DNase can be inhibited by an antibody according to the invention so as to prevent the degradation of chromosomal DNA. On the other hand, this degradation can be promoted by a protein according to the invention, particularly after linkage to a protein which is not considered foreign by the body, e.g. transferrin or BSA, which would be especially suitable for the treatment of tumor cells. The same can be achieved correspondingly with a nucleic acid according to the invention, particularly a DNA which is controlled by a promoter inducible in certain tissues, e.g. tumors, and results in the provision of a protein according to the invention in these tissues after the expression thereof. Moreover, a nucleic acid according to the invention, particularly a DNA, can also be used for inhibiting an above DNase. For this purpose, the nucleic acid is used e.g. as a basis for the preparation of anti-sense oligonucleotides for the expression inhibition of the gene coding for the above DNase.

Thus, the present invention represents a major contribution to the diagnostic and therapeutic detection or registration of apoptosis.

Brief description of the drawing:

Fig. 1 shows the base sequence and the derived amino acid sequence of a protein according to the invention which has DNase activity.

The present invention is explained by the below examples.

Example 1: Preparation and purification of a protein according to the invention

The DNA of fig. 1 was used as template for the preparation of a protein according to the invention. A PCR method was carried out. The primer pair used was: 5'-CAGGGATCCGATGACGATGACAAAATGCACTACCCAACTGCAC-3' and 5'-

GGGGGATCCTCAGGCAGCAGGGCACAG-3'. The PCR supported batch approach and the PCR conditions were as follows:

PCR batch

template DNA (fig. 1)	: 1 µl = 1 ng
Pfu polymerase 10x buffer	: 10 µl = 1 x
DMSO	: 10 µl = 10 %
dNTPs	: 1 µl = 200 µM each
oligonucleotides, 1.5 µl each	: 3 µl = 150 ng each
H_2O bidistilled	: ad 99 µl

PCR conditions

- 92°C - 5 min
- addition of 1 µl Pfu polymerase (Stratagene company) = 2.5 units
- addition of paraffin

PCR

92°C	1 min	
58°C	1 min	1 cycle
72°C	10 min	
92°C	1 min	
58°C	1 min	39 cycles
72°C	2 min	
72°C	10 min	1 cycle

The amplified DNA was cleaved by BamHI and inserted in the only BamHI site of the expression vector pQE-8 (Qiagen company). The expression plasmid pQ/DNaseX was obtained. It codes for a fusion protein comprising 6 histidine residues (N terminus partner) and the protein of fig. 1 according to the invention (C terminus partner). pQ/DNaseX was used for the transformation of E. coli SG 13009 (cf. Gottesman, S. et al., J. Bacteriol. 148, (1981), 265-273). The bacteria were cultivated in an LB medium with 100 µg/ml ampicillin and 25 µg/ml kanamycin and induced with 60 µM isopropyl-ß-D-thiogalactopyranoside (IPTG) for 4 h. Lysis of the bacteria was achieved by the addition of 6 M guanidine hydrochloride. Thereafter, chromatography (Ni-NTA resin)

was carried out with the lysate in the presence of 8 M urea in accordance with the instructions of the manufacturer (Qiagen company) of the chromatography material. The bound fusion protein was eluted in a buffer having pH 3.5. After its neutralization, the fusion protein was subjected to an 18 % SDS polyacrylamide gel electrophoresis and dyed with coomassie blue (cf. Thomas, J.O. and Kornberg, R.D., J. Mol. Biol. 149 (1975), 709-733).

It showed that a highly pure form of a (fusion) protein according to the invention can be prepared.

Example 2: Preparation of an antibody according to the invention

A fusion protein of Example 1 according to the invention was subjected to an 18 % SDS-polyacrylamide gel electrophoresis. After dyeing the gel with 4 M sodium acetate, a 35 kD band was cut out of the gel and incubated in phosphate-buffered common salt solution. Gel pieces were sedimented before the protein concentration of the supernatant was determined by an SDS polyacrylamide gel electrophoresis which was followed by coomassie blue dyeing. Animals were immunized with the gel-purified fusion protein as follows:

Immunization protocol for polyclonal antibodies in rabbits

35 µg of gel-purified fusion protein in 0.7 ml PBS and 0.7 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively, were used per immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
day 14: 2nd immunization (incomplete Freund's adjuvant; icFA)
day 28: 3rd immunization (icFA)
day 56: 4th immunization (icFA)
day 80: bleeding to death.

The rabbit serum was tested in an immunoblot. For this purpose, a fusion protein of Example 1 according to the invention was subjected to an SDS polyacrylamide gel electrophoresis and transferred to a nitrocellulose filter (cf. Khyse-Andersen, J., J. Biochem. Biophys. Meth. 10 (1984), 203-209). The Western blot analysis was carried out as described in Bock, C.-T. et al., Virus Genes 8, (1994), 215-229. For this purpose, the nitrocellulose filter was incubated with a first antibody at 37°C for one hour. This antibody was the serum of the rabbit (1:10000 in PBS). After several wash steps with PBS, the nitrocellulose filter was incubated with a second antibody. This antibody was a monoclonal goat anti-rabbit-IgG antibody linked with alkaline phosphatase (Dianova company) (1:5000) in PBS. Incubation at 37°C for 30 minutes was followed by several wash steps with PBS and then by the alkaline phosphatase detection reaction with developer solution (36 µM 5'-bromo-4-chloro-3-indolylphosphate, 400 µM nitroblue tetrazolium, 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$) at room temperature until bands were visible.

It showed that polyclonal antibodies according to the invention can be produced.

Immunization protocol for polyclonal antibodies in chickens

40 µg of gel-purified fusion protein in 0.8 ml PBS and 0.8 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively, were used per immunization.

Day 0: 1st immunization (complete Freund's adjuvant)
day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
day 50: 3rd immunization (icFA)

Antibodies were extracted from egg yolk and tested in a Western blot. Polyclonal antibodies according to the invention were detected.

Immunization protocol for monoclonal mouse antibodies

12 µg of gel-purified fusion protein in 0.25 ml PBS and 0.25 ml complete Freund's adjuvant and incomplete Freund's adjuvant, respectively, were used per immunization. In the 4th immunization the fusion protein was dissolved in 0.5 ml (without adjuvant).

Day 0: 1st immunization (complete Freund's adjuvant)
day 28: 2nd immunization (incomplete Freund's adjuvant; icFA)
day 56: 3rd immunization (icFA)
day 84: 4th immunization (PBS)
day 87: fusion.

Supernatants of hybridomas were tested in a Western blot. Monoclonal antibodies according to the invention were detected. One of them, 11/78/1, was deposited with DSM under DSM ACC 2211 on April 26, 1995.

Example 3. Detection of the DNase activity of a protein according to the invention

A DNase activity test was made according to the method by Rosenthal, A.L. & Lacks, S.A., Anal. Biochem. 80, (1977), 76-90, with modifications. For this purpose, an 18 % SDS polyacrylamide gel was produced which contained 2 mM EDTA and denatured salmon testis DNA or yeast RNA up to a final concentration of 10 µg/ml in the separation and collection gel. Samples were denatured by boiling them in Laemmli sample buffer, which contained 5 % of 2-mercaptoethanol, for 4 min. A protein according to the invention (from Example 1) and bovine DNase 1 (control) were used as samples. A 10 kd ladder (Gibco BRL company) was used as protein marker, which was separated in the same gel, cut out after electrophoresis and dyed with coomassie blue. For removing the SDS after the electrophoresis, the gel containing the samples was washed with 100 ml 40 mM Tris-HCl, pH 7.6, for 4 x 30 min and incubated in 40 mM Tris-

HCl, pH 7.6, with 0.02 % sodium azide and 2 mM $MgCl_2$, 2 mM $CaCl_2$ and with 2 mM $MgCl_2$, 2 mM $ZnCl_2$, respectively, at room temperature overnight. For detecting the enzymatic activity, the buffer was changed and ethidium bromide was added up to a final concentration of 2 µg/ml. The gel was investigated periodically on a long-wave U.V. light and photographed.

It showed that a protein according to the invention has DNase activity.

Claims

1. A protein having DNase activity, comprising the amino acid sequence of fig. 1 or a functional derivative or fragment thereof.

2. A DNA encoding the protein according to claim 1.

3. The DNA according to claim 2, wherein the DNA comprises:
   (a) the DNA of fig. 1 or a portion thereof,
   (b) a DNA hybridizing with the DNA of (a), or
   (c) a DNA related to the DNA of (a) or (b) via the degenerated genetic code.

4. An expression plasmid comprising the DNA according to claim 2 or 3.

5. A transformant containing the expression plasmid according to claim 4.

6. A process for the preparation of the protein according to claim 1, comprising the cultivation of the transformant according to claim 5 under suitable conditions.

7. Antibodies directed against the protein according to claim 1.

8. Use of the protein according to claim 1 as a reagent for diagnosis and/or treatment.

9. Use of the DNA according to claim 2 or 3 as a reagent for diagnosis and/or treatment.

10. Use of the antibody according to claim 9 as a reagent for diagnosis and/or treatment.

Abstract of the Disclosure

A Protein Having DNase Activity

The present invention relates to a protein having DNase activity, a DNA encoding the same and a process for the preparation thereof. Furthermore, the invention relates to the use of the DNA and the protein as well as antibodies directed against the protein.

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1       CTTGAACGCCTGACCTCGTATCCACCCGCCTCAGCCTCCCAAAGTGCTGGGATTACAGGCATGAGCCACCACGCCCAGCCCATAATTTATTGATTTTT
99      TAAAATTTGTCCAGCCTTCTATTACCACGTCGAATCCATTAGCTACAGCCATCCCATGAGAAGCTGAGTGGATTCAGCCCCACCTCCTGCTCACAGACC
198     CTGTCCGAGCACCTCATTTGTCCCAACAGCATTACTGCAGGACCCCCAGGACGTTGGACTGCCAGCTCCCTGGGTCTCCTCCTCTCTGGGGCAGATCCT
297     CAGTCCTCCCTTGACTTCACGACTGTGGCCAGATCATGTGTGGACTGTCCCTCTCTTTGGGTCTCCAGAGCGCTTGCATCAAACACCCCTAACTCAGAA
396     GTGTGCAGCCACACTGGGACTCAGAACCCAACAACAGGGACAGAAGACTCACGCCCTTGGGGTGCCCGGTCTCGTGGCATCAGGCATGACTTCCAGCTC
495     CTGCGCCTTCCCCAGCAACTGCTGACTGGGGACCCAGACCGGGAGCTGAGCGACGGGCCTGGCGAGCGAAGCTCGGGGTCTCACTCAGGCACCAGCCCC
594     TCCTTGCCCCAGGCTTGAGTGACTCACAGCCCTATTCAGGCAGGAGCTGCTCTTCTGGGGTATCGCGATCCACTTAAGGATGAGGCAGACTTGGTGACA
693     AGCTGGTCTGAGCAGCGCTTCCAGAGCCAGAACTGAGCCCAGTGAGAGCGCACCCTGGAGCAGCCTGGATTCCTGGGGTGTCCCCGGCAGCCACACACA
792     GCCATGCACTACCCAACTGCACTCCTCTTCCTCATCCTGGCCAATGGGGCCCAGGCCTTTCGCATCTGCGCCTTCAATGCCCAGCGGCTGACACTGGCC
            M  H  Y  P  T  A  L  L  F  L  I  L  A  N  G  A  Q  A  F  R  I  C  A  F  N  A  Q  R  L  T  L  A    32
891     AAGGTGGCCAGGGAGCAGGTGATGGACACCTTAGTTCGGATACTGGCTCGCTGTGACATCATGGTGCTGCAGGAGGTGGTAGACTCTTCCGGCAGCGCC
         K  V  A  R  E  Q  V  M  D  T  L  V  R  I  L  A  R  C  D  I  M  V  L  Q  E  V  V  D  S  S  G  S  A    65
990     ATCCCCCTCCTGCTTCGAGAACTCAATCGATTTGATGGCTCTGGGCCCTACAGCACCCTGAGCAGCCCCCAGCTGGGGCGCAGCACCTACATGGAGACG
         I  P  L  L  L  R  E  L  N  R  F  D  G  S  G  P  Y  S  T  L  S  S  P  Q  L  G  R  S  T  Y  M  E  T    98
1089    TATGTGTACTTCTATCGGTCACACAAAACACAGGTCCTGAGTTCCTACGTGTACAACGATGAGGATGACGTCTTTGCCCGGGAGCCATTTGTGGCCCAG
         Y  V  Y  F  Y  R  S  H  K  T  Q  V  L  S  S  Y  V  Y  N  D  E  D  D  V  F  A  R  E  P  F  V  A  Q   131
1188    TTCTCTTTGCCCAGCAATGTCCTTCCCAGCCTGGTGTTGGTCCCGCTGCACACCACTCCTAAGGCCGTAGAGAAGGAGCTGAACGCCCTCTACGATGTG
         F  S  L  P  S  N  V  L  P  S  L  V  L  V  P  L  H  T  T  P  K  A  V  E  K  E  L  N  A  L  Y  D  V   164
1287    TTTCTGGAGGTCTCCCAGCACTGGCAGAGCAAGGACGTGATCCTGCTTGGGGACTTCAATGCTGACTGCGCTTCACTGACCAAAAAGCGCCTGGACAAG
         F  L  E  V  S  Q  H  W  Q  S  K  D  V  I  L  L  G  D  F  N  A  D  C  A  S  L  T  K  K  R  L  D  K   197
1386    CTGGAGCTGCGGACTGAGCCAGGCTTCCACTGGGTGATTGCCGATGGGGAGGACACCACAGTGCGGGCCAGCACCCACTGCACCTATGACCGCGTCGTG
         L  E  L  R  T  E  P  G  F  H  W  V  I  A  D  G  E  D  T  T  V  R  A  S  T  H  C  T  Y  D  R  V  V   230
1485    CTGCACGGGGAGCGCTGCCGGAGTCTGCTGCACACTGCGGCTGCCTTTGACTTCCCCACGAGCTTCCAGCTCACCGAGGAGGAGGCCCTCAACATCAGT
         L  H  G  E  R  C  R  S  L  L  H  T  A  A  A  F  D  F  P  T  S  F  Q  L  T  E  E  E  A  L  N  I  S   263
1584    GACCACTACCCCGTGGAGGTGGAGCTGAAGCTGAGCCAGGCGCACAGCGTCCAGCCTCTCAGCCTCACTGTTCTGTTGCTGCTATCACTCCTGTCCCCT
         D  H  Y  P  V  E  V  E  L  K  L  S  Q  A  H  S  V  Q  P  L  S  L  T  V  L  L  L  L  S  L  L  S  P   296
1683    CAGCTGTGCCCTGCTGCCTGAGCGTCCCCCTACCCCCCCAGGGCCTGCTGCCTTTTGGGACTTAAACCCCAGCCTCCCCCGTCCATCCAGCCCTGGGGC
         Q  L  C  P  A  A  *                                                                                  302
1782    TGGGGGGCTTCAACTATAGTTGCCCTGTGACTGTAGTCCACCCCTGCCTGCCTTGTTTGATTTGGCTCTTGTTCTTTGGTTGGGCTTGTGCCTAGATTA
1881    GGAGAGGAAGCCAGGGGCCCTGCACTCATGCCACCTGCCAGGTAGTGTAGTATCAGGAGTGGAGACAAAGTGGGCTCTGGGTTGGGGTAGGGGAAGGGA
1980    GGGTTCAGAAAGAGGAATGAAGATGTTGTATGACAAGAAGGAAAGTTACTGAGAACAAAAACCCAGATTGGTGAGATAGGACACTTGTGCAGCAGATAT
2079    GCCAATGGGCCATGTTTATTGTGGATGGGTAAGAATCACCAGGAAACCATTAAGCCCCAATAGCTACAAGGAGGGTGGTTAATCTGCTATATCAAACTC
2178    CTTCCCTGAAACCAGCAAACACCGGGAAACATTTTGGCTCATTATAATCCGGTGAACAATGCAGTCAGGCCTGTTATAACCGCTGAGCAGCCACACTCG
2277    CACCTCCTGGGTGCTGTAGTCTGTGTTGGTACAGGCTTCTGCATGCCTGGTAAAGTCCAGCCAAGGCTGGTCAAGGCAACATCTCCACACAGAAAATCT
2376    GCACCAGTTATGTAAGCTAAAAAGCTGTGTGAACCCAGGTGTCCCGGAAAGGGGCTGCAGGACACAGCAAAATGCCAGCAGCGTGCCGGACCCCTCCCT
2475    TCCATCCTCCTCTCCAAAGAACAGAGGTCAGGAAAAACACTGGCTGGGACGCTAGAAGGGTCATGTGTTAACTATAATCACATTTATGGTTTGGAACCA
2574    TCACCCCAAGGTAAAAAAAAAAATAAAAGGTATGTTTGGCAAAATAAAATAAAGGTAATTAAAAACCTAAAAAAAAAAAAAAAAAAAA
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Figure 1

COMBINED DECLARATION AND POWER OF ATTORNEY FOR UTILITY PATENT APPLICATION	Attorney's Docket No. 012627-003

As a below-named inventor, I hereby declare that:
My residence, post office address and citizenship are as stated below next to my name;
I BELIEVE I AM THE ORIGINAL, FIRST AND SOLE INVENTOR (if only one name is listed below) OR AN ORIGINAL, FIRST AND JOINT INVENTOR (if more than one name is listed below) OF THE SUBJECT MATTER WHICH IS CLAIMED AND FOR WHICH A PATENT IS SOUGHT ON THE INVENTION ENTITLED:

DNASE-ACTIVE PROTEIN

the specification of which

(check one) ☐ is attached hereto;

☒ was filed on 10 June 1996 as

International Application No. PCT/DE96/01016

and was amended on _____;
(if applicable)

I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE;

I ACKNOWLEDGE THE DUTY TO DISCLOSE TO THE OFFICE ALL INFORMATION KNOWN TO ME TO BE MATERIAL TO PATENTABILITY AS DEFINED IN TITLE 37, CODE OF FEDERAL REGULATIONS, Sec. 1.56 (as amended effective March 16, 1992);

I do not know and do not believe the said invention was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof or more than one year prior to said application; that said invention was not in public use or on sale in the United States of America more than one year prior to said application; that said invention has not been patented or made the subject of an inventor's certificate issued before the date of said application in any country foreign to the United States of America on any application filed by me or my legal representatives or assigns more than twelve months prior to said application;

I hereby claim foreign priority benefits under Title 35, United States Code Sec. 119 and/or Sec. 365 of any foreign application(s) for patent or inventor's certificate as indicated below and have also identified below any foreign application for patent or inventor's certificate on this invention having a filing date before that of the application(s) on which priority is claimed:

COMBINED DECLARATION AND POWER OF ATTORNEY	Attorney's Docket No. 012627-003

COUNTRY/INTERNATIONAL	APPLICATION NUMBER	DATE OF FILING (day, month, year)	PRIORITY CLAIMED
Germany	195 21 046.8	09 June 1995	YES X NO_
			YES_ NO_

I hereby appoint the following attorneys and agent(s) to prosecute said application and to transact all business in the Patent and Trademark Office connected therewith and to file, prosecute and to transact all business in connection with international applications directed to said invention:

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